

伯氏圆孢多孔菌发酵液化学成分*

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摘要: 从担子菌伯氏圆孢多孔菌 (*Bondarzewia berkeleyi*) 发酵液中分离得 4 个苯并呋喃类化合物 (1~4), 其中一个为新化合物, 其化学结构通过波谱学方法鉴定为: (S)-2-(3-hydroxyisoprop-1-enyl)-2,3-dihydrobenzofuran-5-carbaldehyde (1)。这 4 个化合物均首次从圆孢地花属真菌中分离得到。

关键词: 伯氏圆孢多孔菌; 苯并呋喃; 担子菌

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Dihydrobenzofurans from Culture Broth of the Basidiomycete *Bondarzewia berkeleyi* (Bondarzewiace)

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Abstract: A new dihydrobenzofuran, (S)-2-(3-hydroxyisoprop-1-enyl)-2,3-dihydrobenzofuran-5-carbaldehyde (1) was isolated from the culture broth of *Bondarzewia berkeleyi* together with two known dihydrobenzofurans (2-3) and one known benzofuran (4). Their structures were elucidated on the basis of spectroscopic analysis including 2D NMR experiments, and they were isolated for the first time from the fungi of the genus *Bondarzewia*.

Key words: *Bondarzewia berkeleyi*; Benzofuran; Basidiomycete

Bondarzewia berkeleyi of the family Bondarzewiace (Basidiomycota) grows at the bases or roots of hardwood and deciduous trees of the family Fagaceae (Dai *et al.* 2005). There are no reports on its chemical constituents in literatures. Bernd and co-workers reported a cytotoxic metabolite, montadial A, isolated from the polypore *B. montana* (Bernd *et al.* 1999), they pointed that treatment of these mycelial roots with aqueous KOH causes an intense yellow color. Taxonomically the genus *Bondarzewia* has been placed in

the order Russulales, which is supported by the occurrence of stearyl-velutinal, the chemotaxonomic marker compound for this order (Bernd *et al.* 1999).

In a continuation of our research for biologically active substances from higher fungi (Ding *et al.* 2001; Hellwig *et al.* 2003; Liu, 2002, 2005, 2006; Shao *et al.* 2005; Wang *et al.* 2006), the broth of *B. berkeleyi* was investigated. In this report, we described the isolation and structure elucidation of a new dihydrobenzofuran, (S)-2-(3-hydroxyisoprop-1-enyl)-2,

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3-dihydrobenzo-furan-5-carbaldehyde (**1**), along with two known closely related dihydrobenzofurans, (*S*)-fomannoxin (**2**), (+)-5-formyl-2-(1,2-dihydroxyisopropyl)-2,3-dihydrobenzofuran (**3**), and one known benzofuran, 5-formyl-2-(isopropyl-1-ol) benzofuran (**4**) (Fig. 1).

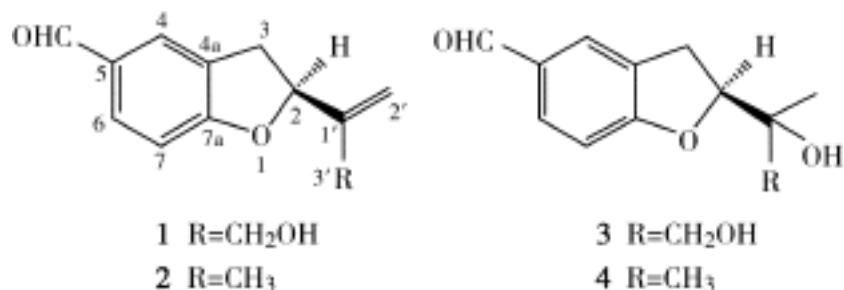


Fig. 1 Structures of compounds 1 - 4

Results and Discussion

Compound **1** was obtained as light yellow oil, and assigned the molecular formula C₁₂H₁₂O₃ based on EI-MS (*m/z* 204, [M]⁺) and HR-TOF-MS ([M+H]⁺ at *m/z* 205.0860; calcd .205.0864). Its IR spectrum revealed the presence of a hydroxyl group (3 441 cm⁻¹) and an aldehyde group (2 854, 2 746 cm⁻¹). The ¹H and ¹³C NMR spectra (Table 1) exhibited an aldehyde signal [_H 9.84 (s); _C 190.6 (d, C = O)], aromatic signals [_H 7.73 (d, *J* = 1.3, H - 4), 7.69 (dd, *J* = 8.2, 1.3, H - 6), 6.91 (d, *J* = 8.2, H - 7); _C 164.8 (s, C - 7a), 133.1 (d, C - 6), 130.8 (s, C - 5), 128.0 (s, C - 4a), 125.9 (d, C - 4), 109.7 (d, C - 7)] appeared as a 1, 2, 4-trisubstituted benzene ring, terminal disubstituted olefinic signals [_H 5.29 (s, H - 2 a), 5.28 (s, H - 2 b); _C 146.8 (s, C - 1), 112.9 (t, C - 2)], a methane signal [_H 5.46 (t, *J* = 8.9, H - 2); _C 84.9 (t, C - 2)], a methoxyl signal [_H 4.29 (d, *J* = 13.7, H - 3 a), 4.25 (d, *J* = 13.7, H - 3 b); _C 63.0 (t, C - 3)], a methylene signal [_H 3.47 (dd, *J* = 15.8, 9.6, H - 3a), 3.21 (dd, *J* = 15.8, 8.2, H - 3b), _C 34.3 (t, C - 3)]. The cross-peaks between H - 2 and H - 3, H - 6 and H - 7 in the ¹H, ¹H-COSY spectra were also shown. These data suggested that compound **1** has a dihydrobenzofuran framework similar to the known compound (*S*)-fomannoxin (**2**). Comparisons of the spectroscopic data of **1** with those of **2** showed that they were strikingly similar except that a methyl (_C 17.1) in **2** was replaced by a hydroxymethyl at _C 63.0 in **1** which corroborated by an IR band at 3 441 cm⁻¹, and implied that **1** was the 3-oxygenated

derivative of **2**. The assignments of **1** were further confirmed by analysis of HMBC correlations (Fig. 2). The absolute configuration of C - 2 in compound **1** was established as *S* by comparison of the optical rotation value of **1** ([_D^{27.9} = +15.2 (*c* 0.048, CHCl₃)) with that of **2** ([_D^{22.7} = +51 (*c* 0.01, CHCl₃)). Thus, the

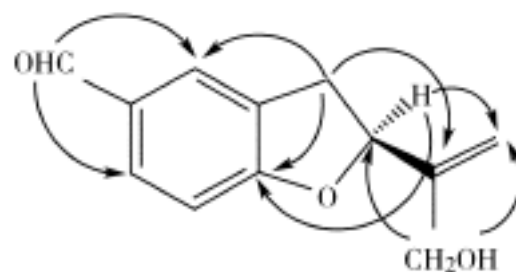


Fig. 2 Key HMBC correlations of **1**

structure of compound **1** was identified as (*S*)-2-(3-hydroxyisoprop-1-enyl)-2,3-dihydrobenzofuran-5-carbaldehyde.

Comparison of the physical and spectroscopic data with the reported data allowed for the identification of compounds **2**, **3**, and **4**, obtained from the above fungus, as (*S*)-fomannoxin (**2**) (Hirotani *et al.* 1977; Kawase *et al.* 1980), (+)-5-formyl-2-(1,2-dihydroxyisopropyl)-2,3-dihydrobenzofuran (**3**), and 5-formyl-2-(isopropyl-1-ol) benzofuran (**4**) (Donnelly *et al.* 1988), respectively. It is noted that the dihydrobenzofuran derivative possessing *S* configuration was only found in fungus (Kawase *et al.* 1980).

Experimental

General experimental procedures Column chromatography (CC): silica gel (200 - 300 mesh, Qingdao Marine Chemical Inc, Qingdao, P. R. China), reversed-phase C₁₈ silica gel (Merk), and Sephadex LH-20 (Amersham Biosciences). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH. Melting point (uncorrected) was determined on an XRC-1 apparatus. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. Optical rotations were measured on a Horiba SEPA-300 spectropolarimeter. IR spectra were obtained with a Tensor 27 spectrometer, with KBr pellets. 1D and 2D NMR spectra were recorded in Bruker AM-400 and DRX-500 spectrometers. MS were registered in a VG Autospec-3000 and API QSTAR Pulsar 1 spectrometers; in *m/z* (rel. %).

Mushroom material and culture The fungus *B. berkeleyi* was collected at the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China, in July 2003, and identified by Prof. Zhu-Liang Yang, Kunming Institute of Botany. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sci-

ences . Culture medium: potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, MgSO_4 1.5 g, citric acid 0.1 g and thiamin hydrochloride 10 mg in 1 l of deionized H_2O (pH 6.5 before autoclaving) . The culture liquid was fermented at 25 for 20 days on a rotary shaker (150 rpm) .

Extraction and Isolation The culture broth (12 liter) was filtered and then successively extracted with AcOEt . The AcOEt extract was evaporated in vacuo and the oily residue (3.0 g) was subjected to CC (RP-18, MeOH/ H_2O (9 1 1 1)) . The fraction (0.3 g) from MeOH/ H_2O (1 1) was further purified by CC (silica gel, petroleum ether/ Me_2CO 12 1), and preparative TLC (petroleum ether/ Me_2CO 9 1, R_f 0.20) to afford the pure compound **1** (3 mg), and preparative TLC ($\text{CHCl}_3/\text{Me}_2\text{CO}$ 30 1, R_f 0.30) to give compound **4** (5 mg) . The fraction (0.7 g) eluted with MeOH/ H_2O (7 3) was further purified by CC (silica gel, petroleum ether/AcOEt 15 1), and preparative TLC (petroleum ether/AcOEt 10 1, R_f 0.23) to give the pure compound **2** (13 mg) .

The fraction (0.3 g) from MeOH/ H_2O (3 7) was further isolated by CC (silica gel, petroleum ether/ Me_2CO 4 1), repeated Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}$ 1 1), and preparative TLC ($\text{CHCl}_3/\text{MeOH}$ 20 1, R_f 0.3) to afford the pure compound **3** (4 mg) .

Compound **1**, $\text{C}_{12}\text{H}_{12}\text{O}_3$, light yellow oil; $[\alpha]_{\text{D}}^{27.9} = +15.2^\circ$ (c 0.048, CHCl_3); UV (CHCl_3) λ_{max} nm (log): 237 (2.79), 294 (3.21); IR $_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3441, 2956, 2925, 2854, 2746, 1656, 1604, 1462, 1109, 950; ^1H and ^{13}C NMR data: see Table = 1; EI-MS: 204 (45, M^+ , $\text{C}_{12}\text{H}_{12}\text{O}_3^+$), 186 (34, $[\text{M}-\text{H}_2\text{O}]^+$), 173 (68), 145 (100), 127 (40), 115 (33), 91 (31), 77 (17); HR-TOF-MS: 205.0860 ($[\text{M}+\text{H}]^+$, $\text{C}_{12}\text{H}_{13}\text{O}_3^+$; calcd . 205.0864) .

Compound **2**, $\text{C}_{12}\text{H}_{12}\text{O}_2$; colorless oil; $[\alpha]_{\text{D}}^{22.7} = +51^\circ$ (c 0.01, CHCl_3); IR $_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3444, 3080, 2856, 2741, 1688, 1606, 1248, 1107, 944; ^1H and ^{13}C NMR data: see Table . 1; ESI-MS: 189 ($[\text{M}+\text{H}]^+$) .

Table 1 ^1H and ^{13}C NMR Data of **1** and **2** (CDCl_3 , in ppm, J in Hz) .

| Pos . | 1 | | 2 | |
|-------|-------------|---|-------------|---|
| | C | H | C | H |
| 2 | 84.9 (d) | 5.46 (t, $J = 8.9$) | 87.2 (d) | 5.30 (t, $J = 8.8$) |
| 3 | 34.3 (t) | 3.47 (dd, $J = 15.8, 9.6$), 3.21 (dd, $J = 15.8, 8.2$) | 33.7 (t) | 3.41 (dd, $J = 15.9, 9.7$), 3.08 (dd, $J = 15.8, 7.8$) |
| 4 | 125.9 (d) | 7.73 (d, $J = 1.3$) | 125.8 (d) | 7.72 (s) |
| 5 | 130.8 (s) | - | 130.5 (s) | - |
| 6 | 133.1 (d) | 7.69 (dd, $J = 8.2, 1.3$) | 133.2 (d) | 7.68 (d, $J = 8.2$) |
| 7 | 109.7 (d) | 6.91 (d, $J = 8.2$) | 109.5 (d) | 6.90 (d, $J = 8.2$) |
| 4a | 128.0 (s) | - | 128.2 (s) | - |
| 7a | 164.8 (s) | - | 165.2 (s) | - |
| 1 | 146.8 (s) | - | 143.1 (s) | - |
| 2 | 112.9 (t) | 5.29 (s), 5.28 (s) | 112.8 (t) | 5.10 (s), 4.95 (s) |
| 3 | 63.0 (t) | 4.29 (d, $J = 13.7$), 4.25 (d, $J = 13.7$) | 17.1 (q) | 1.77 (s) |
| CHO | 190.6 (d) | 9.84 (s) | 190.7 (d) | 9.83 (s) |

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